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THE TRUE NATURE OF THE SO-CALLED "NETTLE-THREADS" OF PARAMŒCIUM.

BY JOHN A. RYDER.

It is well known that if *Paramœcium aurelia* is hurt or irritated in any way that it will discharge from its cuticle what appear to be filaments. This has been known for more than a century, for Elliot in the Philos. Transactions of the Royal Society for 1769, pl. VI, p. 150, gives a very good figure of a *P. aurelia* with these threads projecting from its surface produced by the juice of the horse-shoe geranium. Dr. G. J. Allman in Vol. III of the Quar. Journ. of Microscop. Science (1855), nearly a century later, upon reinvestigating this subject, comes to the conclusion that these discharged threads are of the nature of nettling or defensive organs and that they are discharged from minute oval or elongate cysts that are closely imbedded in the ectosarc just beneath the cuticle of these organisms. Allman also concludes that they are not identical with the cilia which thickly clothe the organism. Various reagents are known to cause this discharge of thread-like processes from the cuticle or surface of *Paramœcium*, but no reagent that I know of does this so effectually as tannic acid, the peculiar potency of which, for this purpose, was first pointed out by H. Warrington (Journ. Roy. Microscop. Soc., 1884).

Most naturalists who have written in recent years upon the structure of the Protozoa have accepted Allman's statement that the threads discharged by *Paramœcium* are not ciliary in nature. Lankester, among others, makes such a statement very positively. Fortunately there are two ways in which to test the truth of this statement.

1. It so happens that if a pretty strong solution of tannic acid is used these threads are entirely detached from the body of *Paramœcium*, if the reagent is allowed to act long enough. Endosmotic action is also thus set up so that the cuticle is lifted off of the underlying ectosarc, which, together with the endosarc, now shrivels into a disorganized, granular mass. The cuticle is seen to be thin and to

be very faintly striated in optic section the striæ extending through it, and closely approximated to one another. The cuticle is smooth and entirely without cilia in this condition, so that a tannic acid solution enables one to completely remove the cilia and also to separate the cuticle from the underlying plasma. The tannic acid acts, in short, as a sort of "depilatory," removing, apparently, every vestige of cilia from the creature. The "threads" thus removed are very long and of nearly the same thickness throughout. They are not much shorter than the short diameter of the body of the creature, but do not taper as do the cilia.

This singular behavior of tannic acid in removing all trace of the presence of cilia from the body of *Paramœcium* has led me to doubt the non-ciliary nature of the threads thrown off by the body of this infusorian. If these threads are not cilia why should there be no trace of cilia left on the cuticle after the prolonged action of a solution of tannic acid? These threads form a dense mat of tangled fibres in the midst of which lie the shriveled remains of its sarcode covered by the cuticle that is now lifted up and everywhere freed from contact with the ectosarc if the creature is subjected to prolonged treatment with tannic acid solution. On *Euplotes*, *Stylonchia* and *Halteria* tannic acid has no such action, proving that these organisms in some way differ very widely in their reaction toward that reagent as compared with the slipper-animalcule. This singular power of tannic acid gives rise to grave suspicions as to the truth of the statement that the "threads" developed by tannic acid on the surface of *Paramœcium* are something different from the cilia. In order to test this suspicion it was necessary to resort to sections of the infusorian in question to see if there really was any ground for the very positive statement of E. R. Lankester and T. J. Parker as to the existence of a fundamental difference between these so-called "nettle-threads" and the cilia of *Paramœcium.*

2. By the aid of a method of entrapping, killing and embedding infusoria and other very small objects in paraffine invented by me and fully described in the American Naturalist, XXIX, 1895, pp. 194–198, it has been possible to obtain serial sections of *Paramœcium,* in quantity, of a thickness not exceeding 2.5 mikrons, $\frac{1}{10\,000}$ inch. These sections so fully reveal the true structure of the ectosarc that nothing further is needed to show how completely this agrees with the view that the "nettle-threads" *are nothing but*

greatly stretched cilia, that are probably extended by some physical action exerted upon them by the tannic acid that has both stretched and swollen them.

The ectosarc of *Paramœcium* is composed entirely of a system of vertical rods of a plasmic substance that stains somewhat more deeply than the endosarc. These rods are cylindrical and densely packed together with but little clear plasma between them. They extend throughout the whole thickness of the ectosarc and compose more than four-fifths of its substance. They are so closely packed together that their arrangement can very readily be made out to correspond to the origins of the cilia from the cuticle. The arrangement of the cilia is quincuncial, that is, in rows in at least two if not in three directions, which correspond with the mode of arrangement or packing together of the rods of denser plasma of the ectosarc just beneath the cuticle.

The sections show that where the cuticle is intact and where reagents like osmic acid or corrosive sublimate have provoked the discharge of the "nettle-threads" there are no cilia; where, on the other hand, there has been no discharge of "nettle-thread" there are cilia present. Intermediate stages can also be sometimes discovered, that is, partially discharged "nettle-threads" may be observed. These facts seem to me to most distinctly prove that the "nettle-threads" and cilia of *Paramœcium* are identical. No one has yet seen these threads discharged except under abnormal conditions. Though I have watched these organisms feeding by the hour I have yet to see that they showed the slightest tendency to throw off the "nettle-threads" except when crushed, roughly handled or brought under the influence of reagents. The fact that few other infusorians have such a distinctly rodded ectosarc, taken together with the fact that other forms are not affected in the same way by tannic acid leads me to conclude in consideration also of the great number of these rods in the ectosarc of *Paramœcium* that they are connected with the cilia individually and are probably only the expression of the same thing as is seen in the rodded or striated ends of many epithelial cells that bear cilia in multicellular animals. At any rate, whatever else can be established by a reinvestigation of this subject by the aid of the methods I have used, one thing is certain, that tannic acid in solutions of almost any strength will finally remove all of the cilia and leave the cuticle smooth especially if allowed to act for half an hour or more. My sections also conclusively prove the ectosarc to be quite thick and composed of uniform, deeply staining rods that are packed as densely in one place as another. The form of these rods as figured by several authors is wholly inaccurate and misleading. For example, they are not fusiform or oval as figured by Lankester in his article Protozoa in the ninth edition of the Encyclopedia Britannica, but cylindrical with bluntly rounded ends. Where the collapsing vesicles ("contractile vesicles" of authors) are placed there appears to be a peculiar separation of these rods at their inner ends to admit of the approximation of the vesicles to the under surface of the cuticle.

For killing and fixing my material I found that osmic acid and corrosive sublimate gave good results, both these reagents acting with such rapidity as to exclude in a large measure the production of artifacts. Staining was done on the slide with hæmatoxylin and Biondi-Heidenhain mixture. Nuclei and food vacuoles are apt to stain strongly if the staining on the slide is attempted with hæmatoxylin. Very good results can also be got by staining the objects *in toto*, as directed in the paper cited above describing my method of handling such very minute objects.